UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

March 23, 2005

MEMORANDUM

Subject: Efficacy Review for Oscar, EPA Reg. No. 4822-539;
DP Barcode: D312173

From: Marcie Wawzysko, Microbiologist Marcie Wawzysko March 23, 2005
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Applicant: S.C. Johnson & Son, Inc.
1525 Howe Street
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Formulation from the Label:

Active Ingredient(s)	**% by wt.**
L-Lactic Acid	2.0%
Other ingredients	98.0%
Total	100.0%

I. BACKGROUND

The product, Oscar, is an Agency registered (Reg. No. 4822-539) sanitizer and fungicide. The applicant has submitted data to support additional label claims as a biofilm sanitizer. Testing was conducted by ATS Labs, located at 1285 Corporate Center Drive, Suite 110 in Eagan, Minnesota; Brain Wave Technologies, Inc. located at 124 Owen Road in Madison, Wisconsin; and by S.C. Johnson & Son, Inc. located at 1525 Howe Street in Racine Wisconsin.

The data package contained a letter to the Agency from the applicant (dated December 7, 2004), the proposed label, and three studies (MRID Nos. 464271-01 to 464271-03) with Statements of No Data Confidentiality Claims for each.

II. USE DIRECTIONS

The product is intended for use as a biofilm sanitizer on hard, non-porous bathroom surfaces in residential, commercial, institutional, and veterinary environments. Surfaces may include cabinets (non-wood), counter tops, sinks, showers, walls, faucets, diaper changing counters, toilets, trash cans, and floors.

The proposed label provides the following instructions for the use of the product as a biofilm sanitizer. Spray surface until thoroughly wet. Let stand for 5 minutes. Then wipe. Reapply as necessary.

The proposed label also includes directions for cleaning, regular surface sanitization and use of the product as a fungicide.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Standard Method for Growing Surface Attached (Biofilm) Bacteria as a Model Biofilm on Glass or Other Smooth Materials for the Purpose of Evaluating the Effectiveness of Antimicrobial Products (as developed by SC Johnson).
The effectiveness of antimicrobial agents is to be determined against model static biofilms on glass carriers. For sanitization, the method has set the measurement of success as a 5 log (99.999%) reduction within 5 minutes of both *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352).

Sanitizer Test (for inanimate, non-food contact surfaces)

The effectiveness of sanitizers for non-food contact surfaces must be supported by data that show that the product will substantially reduce the numbers of test bacteria on a treated surface over those on an untreated control surface. The test surface(s) should represent the type(s) of surfaces recommended for treatment on the label, i.e., porous or

non-porous. Products that are represented as "one-step sanitizers" should be tested with an appropriate organic soil load, such as 5 percent serum. Tests should be performed with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old against *Staphylococcus aureus* (ATCC 6538) and either *Klebsiella pneumoniae* (aberrant, ATCC 4352) or *Enterobacter aerogenes* (ATCC 13048 or 15038). Results must show a bacterial reduction of at least 99.9 percent over the parallel control within 5 minutes. These Agency standards are presented in DIS/TSS-10.

Sanitizing Rinses (for previously cleaned, food-contact surfaces)

Efficacy of sanitizing rinses formulated with halide chemical products including; iodophors, mixed halides, and chlorine bearing chemicals must be substantiated with data derived from the AOAC Available Chlorine Germicidal Equivalent Concentration Method. Data from one test on each of 3 samples, representing 3 different batches, one of which is at least 60 days old, against S. typhi are required. Test results must show product concentrations equivalent in activity to 50, 100, and 200 ppm of available chlorine. (The reference standard is sodium hypochlorite.)

Efficacy of sanitizing rinses formulated with other chemical products including; quaternary ammonium compounds, chlorinated trisodium phosphate, and anionic detergent-acid formulations must be substantiated with data derived from the AOAC Germicidal and Detergent Sanitizers Method. Data from the test on one sample from each of 3 different batches, one of which is at least 60 days old, against both *E. coli* and *S. aureus* are required. When claims for the effectiveness of the product in hard water are made, all required data must be developed at the hard water tolerance claimed. Acceptable results must demonstrate a 99.999% reduction in the number of microorganisms within 30 seconds. The results must be reported according to the actual count and percentage reduction over the control. The minimum concentration of the product which provides the results required above is the minimum effective concentration. Although efficacy must be demonstrated within a contact time of 30 seconds, label claims of less than one minute are not permitted for bacteria. The above Agency standards can be found in DIS/TSS-04 and in Subdivision H - Labeling Guidelines for Pesticide Use Directions - Antimicrobial Products.

Supplemental Recommendations

Antimicrobial agents which claim to be "one-step" cleaner-disinfectants, or cleaner-sanitizers, or agents to be used in the presence of organic soil, must undergo appropriate efficacy testing modified to include a representative organic soil of 5% blood serum. A suggested method to simulate antimicrobial treatment of dry inanimate surfaces is to add the blood serum 5% v/v (19mL bacterial inoculum with 1mL blood serum) to bacterial inoculum prior to carrier contamination and drying. Control data should be produced as described in Supplemental Recommendation 6 of DIS/TSS-2 to confirm the validity of this test with this modification. The suggested organic soil level is appropriate for simulation of lightly to moderately soiled surfaces. For highly soiled surfaces, a prior cleaning step should be recommended on the product label. A suggested procedure for incorporating organic soil load where the antimicrobial agent is not tested against a dry inanimate surface, such as the AOAC Fungicidal Test involves

adding 5% v/v blood serum directly to the test solution (e.g., 4.75 ml test solution + 0.25 ml blood serum) before adding 0.5 ml of the required level (5 X 10^6 /ml) of conidia. These agency standards can be found in DIS/TSS-2.

IV. SUMMARY OF SUBMITTED STUDIES

1. MRID 464271-01 "Additional Efficacy Evaluations for the Registration of Oscar Formulas" by Debra S. Venne. Study conducted by S.C. Johnson & Son, Inc.; Study Number DSV113004. Study completed November 30, 2004.

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352) and *Staphylococcus aureus* (ATCC 6538) in the presence of a 5% organic soil load (fetal bovine serum). Three lots of the product (Samples GLP 472D1, 472D2, and 472D4) were tested according to the Standard Method for Growing Surface Attached (Biofilm) Bacteria as a Model Biofilm on Glass or other Smooth Materials for the Purpose of Evaluating the Effectiveness of Antimicrobial Products. Testing was conducted to pinpoint technical reasons for previous failures on tests performed by Brainwave Technologies personnel. The product was received ready to use. To prepare the biofilm, sterile filter paper was placed on TSB agar plates. Glass slide carriers were inoculated with the 24 hour old culture, incubated at 35±2°C for 15 minutes, then placed inoculated side down onto the filter paper. Six carriers were used per test substance, and six for the Triton X-100 control. Plates and carriers were incubated together for 48±4 hours at 23±2°C. Carriers were lifted from the filter paper with sterile forceps and dried for 40 minutes at 35±2°C. Carriers were sprayed with the test substance 3 to 5 times or until thoroughly wet and held for a contact time of 5 minutes. Following the exposure period, carriers were immersed into 15 mL of D/E Neutralizing Broth then sonicated and vortexed before serial dilution and plating of the broth. Plates were incubated for 24-48 hours in a 35±2°C incubator after which time colony counts were conducted and percent reduction over control calculated. A carrier quantitation control was performed, however controls for sterility, purity, viability, and neutralization were omitted from the test report.

2. MRID 464271-02 "Efficacy Studies for the Registration of Oscar 1" by David Rottjakob. Study conducted by ATS Labs, Inc.; Project Number A02210. Study completed September 2, 2004.

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352) and *Staphylococcus aureus* (ATCC 6538) in the presence of a 5% organic soil load (fetal bovine serum). Three lots of the product (Batches 472D1, 472D2, and 472D4) were tested according to ATS Protocol JW03050903.NFCB.1. The product was received ready to use. To prepare the biofilm, sterile filter paper was placed on TSB agar plates. Glass slide carriers were inoculated with a 10µl loopful of the 24±4 hour old culture and incubated at 35±2°C for 40 minutes, then placed inoculated side down onto the filter paper. Six carriers were used per test substance, and six for the Triton X-100 control. Plates and carriers were incubated together for 48±4 hours at 25.0°C and 50% humidity. Carriers were lifted from the filter paper with sterile forceps and dried for 40 minutes at 36.0°C at a humidity of 58.8%. Carriers were sprayed with the test substance with 3 pumps 6-8 inches away and held for a contact time of 5 minutes. Following the exposure period, carriers were immersed into 15 mL of D/E Neutralizing Broth then sonicated and

vortexed before serial dilution and plating of the broth. Plates were incubated for 44 hours in a 35-37°C incubator after which time colony counts were conducted and percent reduction over control calculated. Controls included those for carrier quantitation, dry carrier control, neutralization confirmation, purity, sterility, and viability.

3. MRID 464271-03 "Efficacy Studies for Oscar 1" by Jean L. Schoeni. Study conducted by Brain Wave Technologies; Project Number 020-04-03-SB-0003. Study completed August 31, 2004.

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352) and *Staphylococcus aureus* (ATCC 6538) in the presence of a 5% organic soil load (fetal bovine serum). Three lots of the product (Batches 472D1 Bottle 91, 472D2 Bottle 17, and 472D4 Bottle 16) were tested according to an unmentioned protocol. The product was received ready to use. To prepare the biofilm, sterile filter paper was placed on TSB agar plates. Glass slide carriers were inoculated with a loopful of the 24±4 hour old culture and incubated at 35±2°C until dry (≥ 15 minutes), then placed inoculated side down onto the filter paper. Six carriers were used per test substance, and six for the Triton X-100 control. Plates and carriers were incubated together for 48±4 hours at 23±2°C. Carriers were lifted from the filter paper and dried for 40±5 minutes at 35±2°C. Carriers were sprayed with the test substance with 3-5 pumps 6-8 inches away, until thoroughly wet, and held for a contact time of 5 minutes. Following the exposure period, carriers were immersed into 15 mL of DE Neutralizing Broth then sonicated and vortexed before serial dilution and plating of the broth. Plates were incubated for 48-54 hours in a 35±2°C incubator after which time colony counts were conducted and percent reduction over control calculated. Controls included those for purity, sterility, numbers control, dry control, and neutralization confirmation.

V. RESULTS

Results of SC Johnson Biofilm Sanitizer Protocol as Performed by Three Laboratories

MRID	Performing Laboratory	Organism	Triton X-100 Log10 Control Count	Log10 Reduction		
				472D1	472D2	472D4
464271-01	SC Johnson	*Staphylococcus aureus*	6.82	>5.65	>5.65	5.39
		Klebsiella pneumoniae	9.02	>7.84	>7.84	>7.84
464271-02	ATS Labs	*Staphylococcus aureus*	7.05	5.96	5.95	5.43
		Klebsiella pneumoniae	7.49	>5.30	>6.34	>7.19
464271-03	Brain Wave Technologies	*Staphylococcus aureus*	7.45	**3.28**	**2.66**	**4.66**
		Klebsiella pneumoniae	>8.45	**<2.10**	**<2.09**	**<3.68**

Results of Confirmatory Studies for Alternate Product Formulations by SC Johnson (MRID No. 464271-01)

Product	*Staphylococcus aureus*- Log Redcution			*Klebsiella Pneumoniae*- Log Reduction		
	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
Triton X-100 (Control Count)	7.07	7.15	7.19	7.86	7.92	8.38
15439H111-1 (Base Formula)	>6.70	>6.78	6.49	>7.49	6.63	>8.01
15439H111-2 (Alternate Fragrance)	6.52	6.61	>6.82	7.05	6.78	>8.01
15439H111-3 (Alternate Fragrance)	>6.70	6.66	>6.82	6.75	7.01	>8.01
15439H111-4 (Alternate Fragrance)	6.14	6.18	>6.82	>7.49	6.32	>8.01

Additional Studies- Comparison of SCJ and Brain Wave Technologies, Inc. cultures (MRID 464271-01)

Product	S. aureus (SCJ Culture)	S. aureus (Brain Wave Culture)	K. pneumoniae (SCJ Culture)	K. pneumoniae (Brain Wave Culture)
Triton X-100 (Control Count 10^x)	7.70	6.63	8.60	8.71
15439H143 (Log Reduction)	>6.52	>5.45	6.64	6.66

Additional Studies- Tester Influence (MRID 464271-01)

Analyst	*S. aureus*- Log Reduction			
	Test 1		Test 2	
	Triton X-100 Control Count 10^x	472D1 Bottle 85	Triton X-100 Control Count 10^x	472D1 Bottle 85
D. Venne	6.71	>6.34	6.07	5.18
J. Kreibich	7.04	>6.68	6.82	>6.46
S. Heathcock	7.01	<6.06	ND	ND
A. Erickson	7.41	6.62	ND	ND
C. Hinkfuss	7.27	>7.70	ND	ND

Analyst	*K. pneumoniae*- Log Reduction			
	Test 1		Test 2	
	Triton X-100 Control Count 10^x	472D1 Bottle 85	Triton X-100 Control Count 10^x	472D1 Bottle 85
D. Venne	8.14	>7.78	7.77	>7.40
J. Kreibich	7.86	>7.49	7.98	>7.61
S. Heathcock	7.85	>7.48	ND	ND
A. Erickson	8.18	>7.81	ND	ND
C. Hinkfuss	8.07	>7.70	ND	ND

VI. CONCLUSIONS

The submitted studies (MRID Nos. 464271-01 through 464271-03) **support*** the use of the product, Oscar, as a biofilm sanitizer for hard, non-porous surfaces which do not undergo routine shear stress (see list of non-allowed surfaces below). The study assigned MRID No. 464271-03 was conducted by Brain Wave Technologies Inc. In this study, all of the trials for each organism failed to obtain a 5 log reduction. SC Johnson conducted several studies in attempt to determine a technical reason for the failures at Brain Wave, testing variables such as tester influence and culture preparation. No conclusive explanation was found. SC Johnson attributes these failures to the lack of experience with GLP's and FIFRA testing at Brain Wave. This was the first GLP study Brain Wave Technologies Inc. had conducted. Subsequently, both SC Johnson's lab and ATS Laboratories performed the testing. Both laboratories produced sufficient passing data based on requirements in the Standard Method for Growing Surface *Attached (Biofilm) Bacteria as a Model Biofilm on Glass or Other Smooth Materials for the* Purpose of Evaluating the Effectiveness of Antimicrobial Products.

VII. RECOMMENDATIONS AND LABELLING

1. The proposed label claims that the product, Oscar, is effective as a sanitizer against 99.999% of biofilm bacteria with a 5 minute contact time. This claim is **acceptable***, only on surfaces that do not undergo routine fluid flow.

   *Static biofilms created as a result of this method are not analogous to those in fluid systems. Fluid flow would likely wash off cells that are loosely attached and easier to kill. It may also provide nutrients to the biofilm, resulting in more EPS and a greater biofilm mass. This leads to the establishment of chemical gradients, a spectrum of phenotypic stages and a community of bacteria that can withstand large concentrations of antimicrobials. Biofilms created by the current method have heightened susceptibility to shear stress and are easier to kill.

   As a result, <u>the following surfaces may not appear on the label</u> <u>as use sites for biofilm sanitization</u>:

   Basins
   Bathroom sink
   Fiberglass tubs, shower surrounds, and sinks
   Plastic and vinyl shower doors
   Shower doors
   Shower fixtures
   Shower walls
   Showers
   Sinks
   Urinals

   The label must state that the product is not for use as a biofilm sanitizer on interior surfaces of sinks, toilets, urinals, or shower parts that routinely undergo fluid stress.

If the applicant desires that these sites remain on the label, they may conduct testing utilizing a drip flow reactor. However, until such data is submitted to the Agency, the afore mentioned sites must be removed from the proposed label.

2. On pages 1 and 2 of the proposed label, claims referring to "grunge" or "slime" are to be removed. These are terms used for non-public health concern biofilms, and are not to be used interchangeably with "biofilms" as tested in the supporting studies.

3. On page 1 of the proposed label, the claim "slows down biofilm build up" must be removed. This claim implies prevention and inhibition of biofilms and has not been tested or proven.

4. On page 1 of the proposed label, change "Seeks and Destroys bacteria and the slime they form" to read "Destroys bacteria and the slime they form." The term "seeks" implies that the agent itself can locate and search for bacteria, this claim has not been tested.

5. On page 2 of the proposed label, eliminate the phrase "Kills bacteria deep down in the biofilms (,) (not just the top layer)." This claim has not been proven; no microscopy has been done to show that several layers of EPS exist, and that the product penetrates through many of them.

6. The bacteria tested in the biofilm protocol (i.e. *Klebsiella pneumoniae* and *Staphylococcus aureus*) need to be indicated on the product label, just as the bacteria tested in the non-food contact surface sanitizer claim are listed.

7. A clear distinction must be made with the layout and wording of the final product label between biofilm sanitization, and surface sanitization of unattached organisms. The contact times for each, 5 minutes and 1 minute respectively, are not to be confused.